

SHORT
COMMUNICATIONS

SNP rs657152 Is Not Associated with the Level of Viral Load in COVID-19 or the Probability of Disease in the Population of Caucasians in Eastern Siberia

E. A. Orlova^a, O. B. Ogarkov^a, *, P. A. Khromova^a, V. V. Sinkov^a, M. A. Khasnatinov^a,
S. N. Zhdanova^a, L. V. Rychkova^a, and L. I. Kolesnikova^a

^a Scientific Center for Family Health and Human Reproduction Problems, Irkutsk, 664003 Russia

*e-mail: obogarkov@sbamsr.irk.ru

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Abstract—Cross-replicating associations with rs657152 at the 9q34.2c locus and rs11385942 at the 3p21.31 locus found in patients with severe COVID-19 in the Caucasian population require the study of the discovered phenomenon in various populations, including as an independent biological marker. Primers and TaqMan probes for PCR discrimination of the A and C alleles in single nucleotide polymorphism (SNP) rs657152 have been developed. The polymorphism of the rs657152 A/C locus was determined in 129 patients with COVID-19 and in a control group of 466 healthy individuals. There were no significant differences in the frequency of distribution of the A and C alleles, 0.47/0.53 and 0.45/0.55, between patients and healthy subjects, respectively. Also, no differences were found in the distribution of alleles in patients with a high viral load in the smear (Ct in the range of 16–25) in comparison with an average and low viral load (Ct in the range of 26–40).

Keywords: COVID-19, rs657152, allele discrimination, PCR, TaqMan probes

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Severe acute respiratory syndrome coronavirus (SARS-CoV-2) was first identified in Wuhan, China, at the end of 2019, and the disease, dubbed COVID-19, quickly became a global pandemic [1]. There is significant variation in the course of the disease among patients infected with the SARS-CoV-2 virus. In the early stages of the pandemic, countries in Europe faced far more COVID-19 cases and deaths than East Asia and Russia. The mechanisms that determine why some populations have more severe cases of the disease and others do not are poorly understood. The pathogenesis of severe forms of COVID-19 and associated respiratory failure has not been adequately studied; however, higher mortality is associated with older age and males [2, 3], a number of cardiovascular diseases and arterial hypertension, obesity, and diabetes [4–6]. Genome-wide association study (GWAS) [7] identified potential genetic factors involved in the development of COVID-19 [8]. A study of 1980 patients from Italy and Spain revealed cross-replicating associations with the rs11385942 variant at the 3p21.31 locus and with rs657152 at the 9q34.2 locus, which were significant at the genome-wide level [8].

The purpose of this work is to develop a PCR test with TaqMan probes suitable for discrimination of alleles A and C of rs657152 and perform a comparative

assessment of the distribution of genotypes in groups of patients with COVID-19 and healthy individuals—representatives of the Caucasian population (Slavs) of Irkutsk oblast.

Compliance with ethical standards. This study was approved by the Ethics Committee of the Scientific Center for Family Health and Human Reproduction Problems. Collection of clinical material (nasal/pharyngeal swabs) was carried out as part of routine anti-epidemic procedures for examining the population of Irkutsk oblast for the presence of the virus SARS-CoV-2. Isolation of RNA/DNA from the samples was carried out with the RealBest extraction kit; PCR diagnostics was conducted with the RealBest RNA SARS-CoV-2 kit. The group of patients included persons with a diagnosis COVID-19 and positive PCR test for the RNA of the virus. The healthy control included contact persons who underwent examination and did not have a positive PCR result for the RNA of the virus or diagnoses of viral respiratory infections or COVID-19. Calculation of the structure and length of primers and probes for discrimination of alleles A and C of rs657152 (Table 1) was produced manually according to the reference nucleotide sequence of the locus *ABO* (<https://www.ncbi.nlm.nih.gov/snp/rs657152>).

Table 1. List of used primers and probes

Primers/Probes	Sequence 5' → 3'	Amplicon length, bp	Annealing, °C
657152F	TCCTACGGGAGGCAGCAGT	103	56
657152R	AATTTAGGACATGTAAAGTTCA		56
657152A	[FAM]TCTCGAATAGCTTCTTGAAAC[A-LNA]G[BHQ1]		66
657152C	[R6G]CTCGAATAGCTTCTTGAAAC[C-LNA]G[BHQ1]		66

Table 2. Characteristics of the surveyed samples

Indicator	Control	Patients with COVID-19
<i>N</i>	<i>N</i> = 466	<i>N</i> = 129
Men, % (<i>N</i>)	33.0 (154)	41.1 (53)
Women, % (<i>N</i>)	67.0 (312)	58.9 (76)
Age, years	41.0 ± 16.1	38.8 ± 21.5
Genotype rs657152 AA	101	34
Genotype rs657152 AC	215	54
Genotype rs657152 CC	150	41
Allele A	417 (0.45)	122 (0.47)
Allele C	515 (0.55)	136 (0.53)
Deviation from the Hardy–Weinberg equilibrium	<i>P</i> > 0.05	<i>P</i> > 0.05
PCR threshold value (Ct) for detection SARS-CoV-2	–	35.0 ± 5.5

The synthesis of primers and probes was carried out by Lumiprob RUS LLC. PCR was performed in the variant with real-time detection (RT-PCR) on a CFX amplifier (BioRad) using BioMaster HS-qPCRHi-ROX (BIOLABMIX LLC) and equimolar concentrations of primers (0.5 μM) and probes (0.1 μM), respectively. Cycling regime: 95°C for 5 min; 40 cycles, 95°C for 10 s, 55°C for 10 s; 72°C for 10 s. The calculation of statistical data, including the assessment of the deviation from the Hardy–Weinberg equilibrium, was carried out in Excel tables (Table 2); the deviations were statistically insignificant.

The initial objective of the study was to create two PCR tests to detect the cross-replicating association rs657152 at the 9q34.2c locus and rs11385942 at the 3p21.31 locus [8]. Unfortunately, detection of the GA/G insertion–deletion at the 3p21.31 locus (rs11385942) by the TaqMan PCR test turned out to be technically difficult (data not shown), and the discrimination of alleles in the second test was not achieved. As a result, the study was carried out only for SNP rs657152.

The groups of patients and healthy controls did not differ significantly in sex and age. As one can see from Table 2, the volume of the obtained samples made it possible to achieve a ratio of genotypes that did not differ significantly from that expected according to Hardy–Weinberg. Analysis of the results obtained did not reveal any differences in the distribution of genotypes or alleles between the group of patients with

COVID-19 and the group of healthy individuals. We also failed to find significant differences in the distribution of alleles in COVID-19 patients with a high viral load in the smear (Ct in the range of 16–25) compared with COVID-19 patients with an average and low viral load (Ct in the range of 26–40).

Comparison of the obtained allele frequencies with the data of the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/rs657152#frequency_tab) revealed significant differences ($\chi^2 = 13.2$, $P < 0.001$) of the studied control group in the frequency distribution of the A and C alleles (0.45/0.55) in relation to the global (0.39/0.61) distribution. A similar comparison of the distribution of the A and C alleles (0.45/0.55) with the Caucasian population (0.37/0.63) also showed significant differences ($\chi^2 = 23.1$, $P < 0.001$). It should be noted that the A allele of rs657152 is characterized as the “COVID-19 risk allele” together with the GA variant of rs11385942 [8]. The results obtained on the distribution of the A allele of rs657152 are the closest (geographically and ethnically) to its distribution in Estonia (0.44) and Finland (0.47) [8]. The study of the distribution of the rs657152 alleles on the territory of Russia, most likely, has not yet been carried out.

Thus, the identification of allele A in the analysis of SNP rs657152 in the population of the Caucasian inhabitants (Slavs) of Irkutsk oblast alone cannot be considered a risk factor for a more severe course of COVID-19.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in a study involving people comply with the ethical standards of the institutional and/or national committee for research ethics and the 1964 Helsinki Declaration and its subsequent changes or comparable ethical standards.

Informed voluntary consent was obtained from each of the participants enrolled in the study.

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